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Scientists show that Resistance to Aminoglycoside Antibiotics is Due in Part to Target Mimicry

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Bacterial resistance to antibiotics is a serious public health problem. The emergence of "superbugs" — pathogenic bacteria that can survive the effects of most commonly used antibiotics — significantly compromises existing treatments against infectious diseases. The predominant mechanism of resistance to aminoglycosides, a class of bactericidal antibiotics that are widely used in hospitals, is enzyme-catalyzed chemical modification of the drug. One of the enzymes that catalyze antibiotic detoxification is aminoglycoside kinase (3') type IIIa [APH(3')-IIIa]. Researchers from McGill University have obtained three-dimensional structures of this enzyme with two structurally diverse aminoglycoside antibiotics bound in the active site. These structures provide insight into how the enzyme is able to detoxify many different aminoglycosides, and suggest novel treatments.

Due to the widespread use and misuse of antibiotics, bacteria have developed mechanisms for evading the effects of these drugs. Enzymatic modification of the antibiotic is by far the major antibiotic resistance mechanism to aminoglycoside antibiotics. Ordinarily, an aminoglycoside exerts its bactericidal effect by binding to the A-site of the 16S ribosomal RNA, resulting in errors in translation, and ultimately bacterial cell death. But an aminoglycoside altered through enzymatic modification has dramatically reduced affinity for the ribosomal RNA, and is therefore no longer harmful to the pathogenic bacteria.

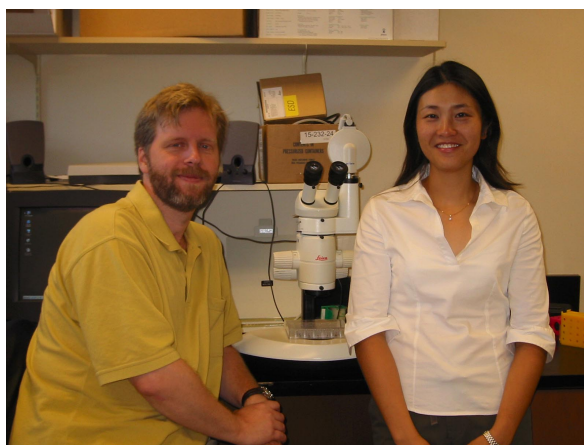
APH(3')-IIIa in complex with adenosine diphosphate (ADP) and either kanamycin or neomycin. These two crystal structures represent the first aminoglycoside kinase structures with bound antibiotic substrates.

Crystals were obtained by co-crystallization of the enzyme in the presence of ADP and kanamycin or neomycin. We collected 2.9Å resolution data for the kanamycin ternary complex from a rotating copper anode x-ray generator. We also collected 2.4Å and 2.7Å resolution data sets for ternary complexes of a second kanamycin and neomycin, respectively, at the X8C

beamline of the National Synchrotron Light Source at Brookhaven National Laboratory.

Our results reveal how the three-dimensional structure of APH(3')-IIIa is able to bind to various aminoglycoside substrates. First, since aminoglycosides are invariably positively charged molecules, the substrate-binding pocket is lined with numerous negatively charged residues. Secondly, the APH(3')-IIIa antibiotic binding pocket is an extended area which has three sub-sites, labeled A, B and C, and different types of aminoglycosides bind to either sub-sites A and B or A and C. Finally, a flexible loop forms one wall of the antibiotic binding pocket, allowing for additional variability in aminoglycoside binding.

We studied aminoglycoside kinase (3') type IIIa [APH(3')-IIIa], an enzyme that can be found in several pathogenic bacteria, including *enterococci* and *staphylococci*. This enzyme can inactivate at least ten structurally diverse aminoglycosides by transferring one or two phosphate groups from adenosine triphosphate (ATP) to these drugs. We have studied the crystal structure of



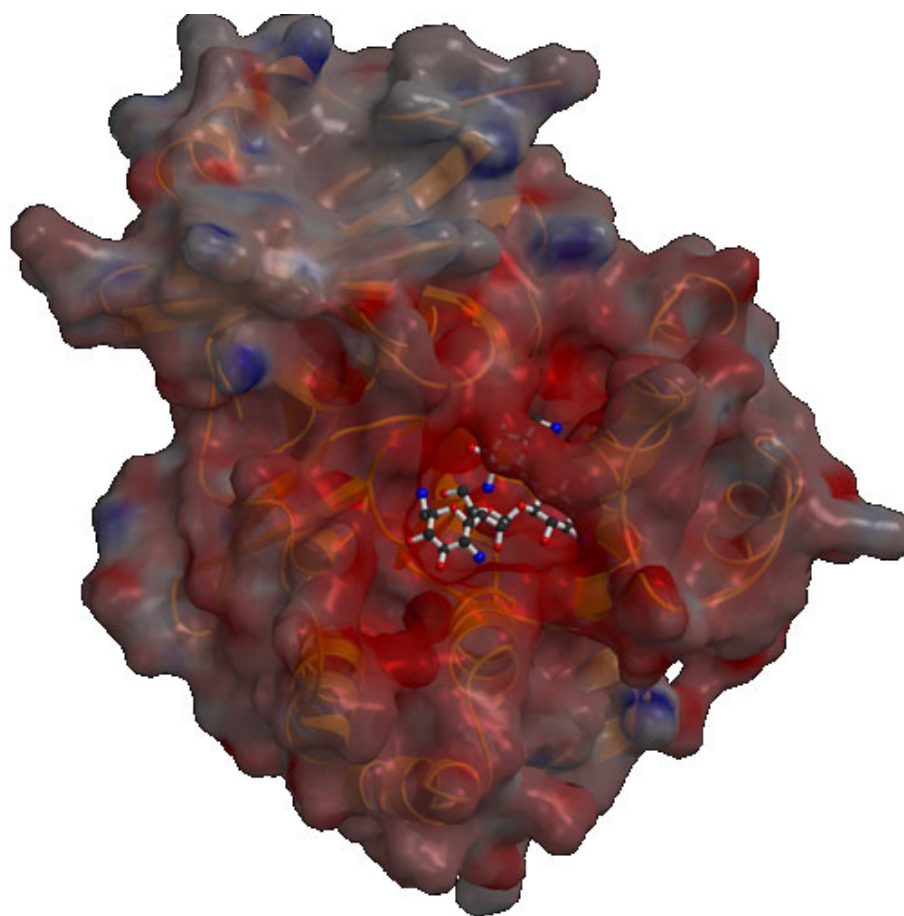
Albert Berghuis (left) and Desiree H. Fong

To understand why APH(3')-IIIa provides resistance to aminoglycosides, we compared the A-site of the bacterial ribosome with APH(3')-IIIa. We noticed that the binding pocket of APH(3')-IIIa successfully mimics the aminoglycoside-bound A-site of the ribo-

some. Although they differ in overall structure and in the nature of the polypeptide (i.e. amino acids vs. ribonucleic acids), they display identical spatial arrangement of hydrogen bond donor and acceptor groups. This means that aminoglycosides that bind to the A-site of the ribosome may also bind to APH(3')-IIIa, which acts as an efficient decoy target for aminoglycosides.

The close mimicry of the bacterial ribosomal A-site by APH(3')-IIIa raises concerns about the development of new antibiotics that target the 16S RNA. Fortunately, the antibiotic binding pocket of APH(3')-IIIa and the ribosomal A-site differ in one crucial aspect: They display significantly different van der Waals interactions with aminoglycosides. This difference suggests possible strategies for the design of novel

variant aminoglycosides that can interact with the ribosome but cannot be detoxified by APH(3')-IIIa and related antibiotic resistance factors.



Ribbon diagram of aminoglycoside kinase (3') type IIIa [APH(3')-IIIa] in complex with the aminoglycoside antibiotic, Neomycin. The antibiotic is shown in ball and stick style and the molecular surface of APH(3')-IIIa is displayed as a semi-transparent representation colored according to the electrostatic potential (red being negatively charged and blue being positively charged).